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Effect of irradiance and spectral composition of radiation on *in vitro* shoot proliferation in *Malus domestica* Borkh.

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Abstract

Four clones of *Malus domestica* Borkh. cv. Golden Delicious - namely Smoothee, Crielaard, Reinders and Golden B - were cultured *in vitro* from single-node microcuttings placed on solid medium under irradiance (PPFD) of 50 μmol m^{-2} s^{-1}. After 9 months an average shoot proliferation of 5.3 was achieved; Crielaard showed the highest rate (7.1), followed by Golden B (5.4), Smoothee and Reinders (4.4). Proliferating shoots were then exposed to higher PPFD (80 μmol m^{-2} s^{-1}) and different spectral composition of radiation using PMMA-B and PMMA-R/FR filters. High PPFD decreased the average proliferation rate to 4.5, in particular in Crielaard and Golden B, while it increased proliferation in Reinders. When a PMMA-R/FR filter was interposed, the mean proliferation rate slightly increased. PMMA-B filters decreased the overall proliferation rate to 3.0; only in Crielaard it was increased, but shoots were very small. Thus PPFD and spectral composition influenced *in vitro* shoot proliferation and growth and the responses were different among the clones.

Additional key words: apple tree, micropropagation, photomorphogenesis.

Introduction

It is very hard to change irradiance in the field without changing other climatic factors - *e.g.* temperature - and thus it is still not clear whether and how much, light spectral composition and/or irradiance actually influence apple-tree and fruit growth. Since it is much easier to control the climatic factors in a growth chamber, the present research on the role of irradiance was carried out *in vitro* as a contribution to field experiments.

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Abbreviations: B - blue; FR - far red; IR - infra red; PMMA - polymethylmethacrylate; PPFD - photosynthetic photon flux density; R - red.

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Micropropagation of apple-trees have been reported for both rootstocks (Simmonds 1983, Famiani *et al.* 1994) and scion cultivars including cv. Golden Delicious (Webster *et al.* 1985, Larsen and Higgins 1993). However, very few studies have been carried out on their response to different *in vitro* environmental conditions.

The aim of the research was to find out whether selected genotypes of Golden Delicious *in vitro* would respond differently to PPFD and spectral composition of radiation.

Materials and methods

Explants were taken from 8-year-old apple-trees (*Malus domestica* Borkh. cv. Golden Delicious). Four clones - Crielaard, Reinders, Smoothee and Golden B - with different fruit shape and susceptibility to russeting were cultivated at the Experimental Station of Laimburg (Bozen, Italy). Explants were taken both in the growing season (in May and June and later in July and September from fast-growing shoots) and in February, after vernalization. They were surface-sterilized for 2 min with aqueous solution of 0.2 % $HgCl_2$ and finally cut into 15 mm long single-node axillary bud microcuttings.

The microcuttings were aseptically placed in test tubes containing 15 cm^3 of the solid culture medium PM (Fiorino and Leva 1981) with the following additions: 30 g dm^{-3} of sucrose, 6 g dm^{-3} of agar and 1 mg dm^{-3} of BA (benzyladenine). The pH was adjusted to 5.5 with 0.1 M KOH.

Once established *in vitro*, proliferating shoots were cultured in plastic boxes containing 100 cm^3 of culture medium and subcultured every 25 d. Shoots were grown in growth chamber under PPFD of 50 μmol m^{-2} s^{-1} (*Philips TLD 36W/33* fluorescent tubes), 16-h photoperiod and temperature of 24 ± 1 °C.

At the end of a multiplication cycle - about 25 d, selected single 25 - 30 mm long (3 - 4 nodes on average) shoots were subcultured in fresh medium and exposed to 4 treatments: *1*) PPFD 80 μmol m^{-2} s^{-1} (supplied by *Osram HQL-R 400W* lamps), unfiltered; *2*) PPFD 80 μmol m^{-2} s^{-1}, PMMA-B filter; *3*) PPFD 80 μmol m^{-2} s^{-1}, PMMA-R/FR filter and *4*) PPFD 50 μmol $m^{-2}s^{-1}$, unfiltered. PMMA-B filter was capable of cutting wavelengths shorter than 520 nm in order to avoid UV-B, violet and blue light and thus interfering with UV-B/B photoreceptors; PMMA-R/FR-filter was capable of cutting wavelengths shorter than 400 nm and between 650 and 760 nm in order to avoid UV-B, R and FR light, where phytochrome is active with both the primary (Pr 666 nm and Pfr 730 nm) and the secondary (380 nm) peaks of absorbance. The irradiance was measured with a *LI-1000* (*Licor*, Lincoln, USA) quantum meter.

To evaluate the plant response to irradiance, explant proliferation (number of shoots) and growth (total length of the shoots and fresh mass) were measured after 30 d; mean length of the shoots was calculated. Eight shoots of each of the 4 clones were exposed to the 4 treatments, for a total of 128 shoots. The experiment was repeated 5 times for a total of 640 shoots observed.

Results and discussion

In vitro cultures of Crielaard, Reinders, Smoothee and Golden B clones were established by taking microcuttings from early developing sprouts from hardwood shoot collected in February, differently from Perleberg 3, another Golden Delicious clone, where explants were collected from the apical portion of the shoots in the growing season (De Paoli 1979). The genotypes performed differently *in vitro* (Table 1). Considering the overall performance, Crielaard showed the highest proliferation rate and lowest mean length of the shoots. Smoothee and Reinders shoots showed the highest mean length and growth rate of the shoots. Golden B was the least vigorous *in vitro,* however it performed better than Perleberg 3 on Jones medium (Jones *et al.* 1977), which showed a proliferation rate of 2.7 (De Paoli 1979).

Table 1. Average shoot proliferation and growth of Golden Delicious clones after 30 d. Values with different letters are significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

Clones	Number of shoots [explant $^{-1}$]	Total shoots length [mm explant $^{-1}$]	Mean shoot length [mm]
Crielaard	5.2 a	59.2 bc	11.6 c
Smoothee	3.9 b	69.1 a	17.5 a
Reinders	3.9 b	66.5 ab	17.8 a
Golden B	4.2 b	53.0 c	13.5 b

Treatments significantly influenced number, mean length and total length of the shoots (Table 2) confirming the hypothesis that cv. Golden Delicious respond to irradiance (Noè and Eccher 1996) and that selected clones respond differently. Under lower PPFD, Crielaard showed the highest proliferation rate and the lowest mean length of the shoots, while Reinders and Smoothee showed higher mean and total shoot length. Although the shoot growth was the lowest, Golden B resulted in the highest shoot mass because of shoot thickness (Fig. 1). No relevant formation of callus was observed at the shoot base.

Shoot exposure to higher PPFD (unfiltered) decreased overall proliferation, as already observed in other woody species (Noè and Eccher 1994). Smoothee (Fig. 2) and Reinders (Fig. 3) benefitted most from the PPFD increment, increasing the production of fresh mass per shoot by 70 % and 95 %, respectively, and reaching the highest mean and total shoot length over all treatments and clones (Table 2). Reinders produced abundant callus at the shoot base and very large leaves (Fig. 3). Crielaard (Fig. 4) did not perform as well and recorded a consistent decrease in proliferation rate and shoot growth.

Under PMMA-R/FR-filter the overall proliferation rate further slightly increased. The change in activity of the axillary buds was probably caused by the interaction between light spectral composition and cytokinins, which was observed *e.g.* by Baraldi *et al.* (1988). The response of each clone was very consistent: the proliferation rate increased in Crielaard and Golden B, while it decreased slightly in Smoothee and considerably in Reinders (Table 2). Golden B reached its best among

the treatments as far as proliferation and total and mean shoot growth are concerned, however, fresh mass was very low, indicating thinner shoots and absence of callus (Fig. 1). Smoothee (Fig. 2) and Reinders (Fig. 3) showed reddening of leaves.

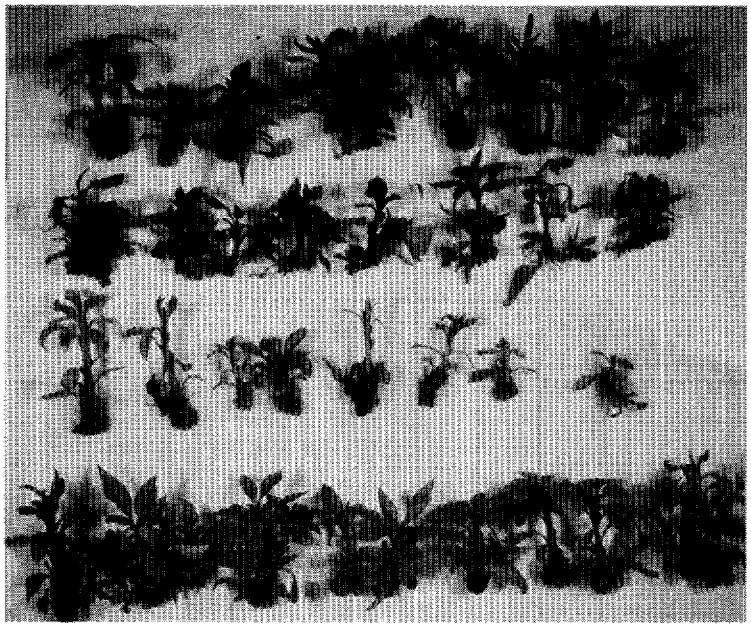
Table 2. Shoot proliferation (number of shoots) and growth (shoot length and fresh mass) of Golden Delicious clones, after 30 d of exposure to 4 light treatments. Means ± standard deviation obtained from 40 explants in 5 repeated experiments except for fresh mass (mean of 3 observations).

PPFD [μmol m^{-2} s^{-1}]		Clone	Shoot number [explant $^{-1}$]	total length [mm explant $^{-1}$]	mean length [mm]	fresh mass [mg explant $^{-1}$]
50	unfiltred	Crielaard	5.8 ± 2.7	61.2 ± 37.0	10.8 ± 4.5	290
		Smoothee	4.4 ± 1.9	72.9 ± 32.8	17.3 ± 4.8	360
		Reinders	4.3 ± 1.8	72.0 ± 3.55	17.2 ± 5.6	290
		Golden B	4.7 ± 2.3	54.5 ± 30.3	11.7 ± 3.0	390
80	unfiltered	Crielaard	4.2 ± 2.1	49.6 ± 34.3	11.9 ± 5.1	275
		Smoothee	4.8 ± 2.0	99.0 ± 53.3	20.6 ± 6.3	600
		Reinders	4.7 ± 2.0	90.2 ± 40.5	20.2 ± 6.8	560
		Golden B	4.1 ± 1.8	49.3 ± 23.9	12.9 ± 4.6	440
80	PMMA-B	Crielaard	5.7 ± 2.7	65.0 ± 34.8	11.7 ± 6.4	300
		Smoothee	2.1 ± 0.9	30.6 ± 12.6	15.5 ± 5.2	190
		Reinders	2.7 ± 2.1	44.7 ± 35.6	17.9 ± 6.3	250
		Golden B	2.8 ± 1.8	32.6 ± 14.6	14.2 ± 6.5	210
80	PMMA-R/FR	Crielaard	5.3 ± 2.8	61.4 ± 38.6	12.1 ± 5.0	340
		Smoothee	4.5 ± 2.6	74.0 ± 51.6	16.4 ± 4.9	260
		Reinders	3.9 ± 2.0	59.2 ± 28.3	16.0 ± 5.4	400
		Golden B	5.1 ± 2.5	75.6 ± 41.1	15.5 ± 5.5	200

Under PMMA-B filter the proliferation rate decreased, as did fresh mass production (Table 2); shoot growth was generally decreased (Figs. 1 - 4). In all genotypes, this treatment caused production of abundant callus at the shoot base and formation of adventious shoots from callus in Golden B and Reinders. Only Crielaard partially benefitted from this spectral composition, reaching its highest shoot mean length and growth among the treatments. The other clones performed very badly except for shoot mean length.

The results of the experiments here reported reveal different photomorphogenetic responses *in vitro* of different genotypes within the same Golden Delicious cultivar. PPFD and spectral composition influenced *in vitro* shoot proliferation and growth and the response was different among the clones. Although these experiments cannot be considered as directly supporting the hypothesis that fruit shape and susceptibility to russeting in different clones and at different altitudes are influenced by light condition (Noè and Eccher 1996), this research demonstrates that also *in vitro* the different Golden Delicious genotypes are sensitive to spectral composition of radiation and that there is an interaction between irradiance and genotype in determining growth responses. These results encourage further research

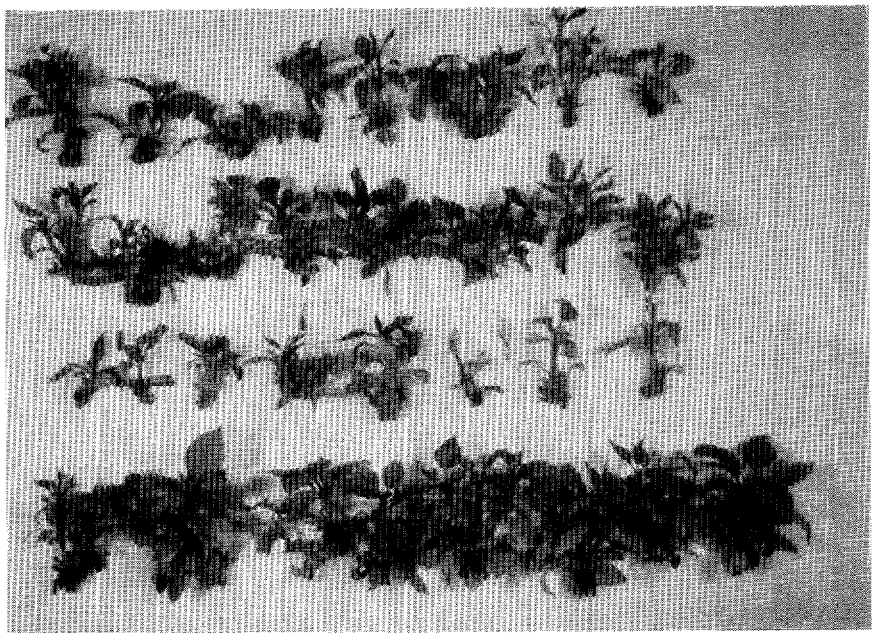
1



2

Figs. 1 and 2. From top to bottom: shoots of Golden delicious clones grown for 30 d under unfiltered irradiance of 50 μmol m^{-2} s^{-1}, irradiance of 80 μmol m^{-2} s^{-1} filtered by PMMA-R/FR, irradiance of 80 μmol m^{-2} s^{-1} filtered by PMMA-B and unfiltered irradiance of 80 μmol m^{-2} s^{-1}. Clones: Golden B (Fig. 1), Smoothee (Fig. 2.).

3



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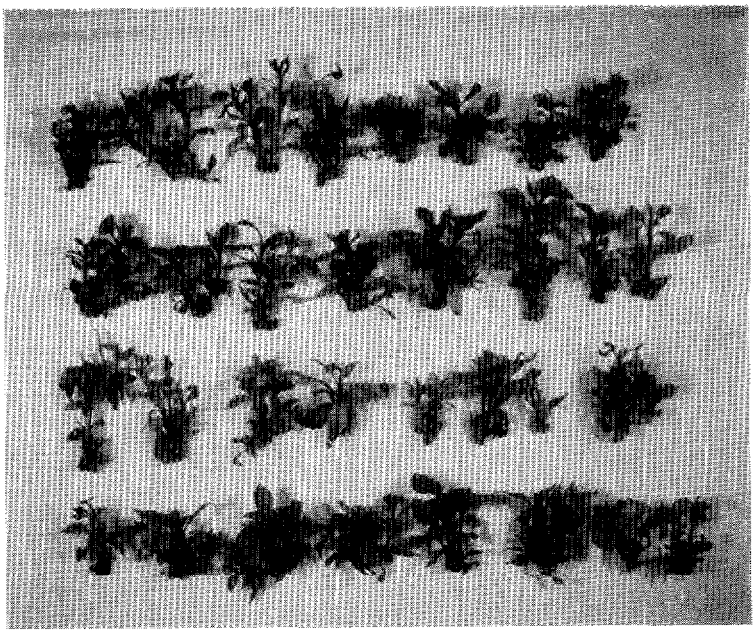

Figs. 3 and 4. From top to bottom: shoots of Golden delicious clones grown for 30 d under unfiltered irradiance of 50 μmol m^{-2} s^{-1}, irradiance of 80 μmol m^{-2} s^{-1} filtered by PMMA-R/FR, irradiance of 80 μmol m^{-2} s^{-1} filtered by PMMA-B and unfiltered irradiance of 80 μmol m^{-2} s^{-1}. Clones: Reinders (Fig. 3) and Crielaard (Fig. 4).

on the role of irradiance in determining 'Golden Delicious' clone responses at different altitudes.

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